Sera from 60 patients with pernicious anemia were studied for the presence of lymphocytotoxins (LCT), blocking and binding autoantibodies to intrinsic factor, and gastric parietal cell autoantibody. LCT were found in 21 sera. Cytotoxic activity was detected at 15°C but not at 24°C and did not appear to have HL-A specificity. Autocytotoxins were present in four of eight patients tested. Blocking antibody to intrinsic factor was found in 34 sera, binding antibody in 14 sera, and parietal cell antibody in 48 sera. Sera from 14 patients contained all three types of autoantibodies, and 12 sera were void of these autoantibodies. Of the 14 sera with three types of autoantibodies, LCT were detected in ten; none of the 12 sera without autoantibodies showed cytotoxic activity. These studies suggest that LCT may reflect the degree of autoimmune derangement in pernicious anemia; alternatively, LCT may represent naturally occurring immunosuppressants.

Cytotoxic antibodies against lymphocytes have been detected in the sera of women immunized by pregnancy, in homograft recipients, and in certain viral infections.1,2 Lymphocytotoxins (LCT) have recently been found in certain autoimmune disorders, including systemic lupus erythematosus and rheumatoid arthritis.3 The term autoimmune disease implies that the initiation and/or perpetuation of the disease process is mediated by immune mechanisms. For unknown reasons, LCT found in patients immunized by allogeneic lymphocytes appear to have greatest activity at 24°C, whereas those present in viral and autoimmune disorders react best at 15°C.2 Pernicious anemia has also been considered to be an autoimmune disease by some workers, since the sera and/or gastric juices from the vast majority of these patients contain autoantibodies to the gastric parietal cell and to intrinsic factor.4 Other investigators have suggested that the autoimmune phenomena found in pernicious anemia are of secondary importance.5 Because of the apparent association between LCT and autoimmunity, a study was undertaken to determine the frequency and type of LCT in pernicious anemia.

MATERIALS AND METHODS

Sera were obtained from 60 patients with pernicious anemia. The diagnosis was established by Schilling's tests performed with and without hog intrinsic factor; almost all patients had been in remission for a minimum of 5 yr and were receiving intermittent injections of vitamin B12. The group consisted of 34 males and 26 females with an age...
range of 36–68 yr. Sera were examined for lymphocytotoxic activity, blocking antibody to intrinsic factor (IF), binding antibody to the complex of IF and vitamin B₁₂, and antibody to gastric parietal cells. LCT were measured at 24°C and 15°C by a microdrop technique using panels of lymphocytes from 25 to 39 random donors. These panels possessed most of the known HL-A antigens. The test was performed by incubating 0.001 ml of serum with 0.001 ml of a 10⁶/ml suspension of lymphocytes for 30 min, followed by addition of 0.005 ml of rabbit serum. After 1 hr, cytotoxic activity was detected by exclusion of eosin dye. Blocking antibody to IF was determined by the charcoal assay of Gottlieb et al. and the results expressed as nanograms of IF-mediated vitamin B₁₂ cobalt-60 uptake blocked by 0.1 ml of serum. Sera were examined for binding antibody to IF by a radi immuno-diffusion technique and for antibody to parietal cells by indirect immunofluorescence using fluorescence conjugated rabbit antiserum to human IgG and rat gastric mucosa as substrate. The χ² method was used to determine if a significant correlation existed between the presence of LCT and the three types of autoantibodies described above.

RESULTS

LCT were present in 21 of 60 sera; 13 of the sera were from males and eight were from females. These cytotoxins were detected when the test was performed at 15°C but not at 24°C. LCT appeared to be directed against non-HL-A antigens present on the lymphocyte and reacted with lymphocytes from a minimum of two to a maximum of 18 random donors. Eight of the sera containing LCT were tested against autologous lymphocytes, and four showed autocyto toxic activity. Blocking antibody to IF was found in 34 sera, binding antibody in 14 sera, and parietal cell antibody in 48 sera. The frequency of these autoantibodies was similar to that reported previously in large groups of patients with pernicious anemia. Sera from 14 patients contained three types of autoantibodies, and 12 sera were void of these antibodies. Of the 14 sera containing all three types of autoantibodies, ten had LCT (71%); cytotoxic activity was significantly associated with the presence of three different autoantibodies in the same serum, the p value being 0.005. A similar correlation (p=0.005) existed between LCT and binding antibodies to IF, since the sera with binding antibody also contained blocking and parietal cell antibody (Table 1). No association was seen between LCT and blocking or parietal cell antibody, or between LCT and the titers of the respective autoantibodies. Lymphopenia was not present in those patients with LCT. Cold-reactive LCT were present in the sera of nine of 80 healthy adults (11%); these LCT usually showed weak cytotoxic activity against limited numbers of random donor lymphocytes.

Table 1. Association of Lymphocytotoxins and Autoantibodies to Intrinsic Factor and Parietal Cells in Pernicious Anemia Sera

<table>
<thead>
<tr>
<th>Type of Autoantibody Present in Serum</th>
<th>No. Sera</th>
<th>Autoab+</th>
<th>Autoab±</th>
<th>Autoab−</th>
<th>LCT+</th>
<th>LCT−</th>
<th>p Value (From χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocking antibody to IF</td>
<td>34</td>
<td>15</td>
<td>19</td>
<td>6</td>
<td>20</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>Binding antibody to IF</td>
<td>14</td>
<td>10</td>
<td>4</td>
<td>11</td>
<td>35</td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>Parietal cell antibody</td>
<td>48</td>
<td>16</td>
<td>32</td>
<td>5</td>
<td>7</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>Three types of autoantibodies</td>
<td>14</td>
<td>10</td>
<td>4</td>
<td>11</td>
<td>35</td>
<td></td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Autoab, autoantibody to intrinsic factor or parietal cells.
†LCT, lymphocytotoxin.
Positive, +; not detected, ---
DISCUSSION

LCT have been described in three disorders thought to be autoimmune; these include systemic lupus erythematosus, rheumatoid arthritis, and pernicious anemia. Four possible mechanisms might be responsible for the production of LCT in autoimmune states. First, LCT could act as natural immunosuppressants, since lymphocytes, either by production of autoantibodies or by their role in cellular immune responses, are the prime cells involved in mediation of autoimmunity. Second, LCT may be a nonspecific reflection of a hyperimmune state or deranged immune response. Third, persistent viral infection has been postulated in certain autoimmune diseases, and LCT have been shown to be associated with certain viral infections. Fourth, LCT may represent cross-reacting antibodies, i.e., antibodies induced by antigenic determinants common to several tissues.

LCT were particularly prevalent in those patients with pernicious anemia whose sera contained three types of autoantibodies, blocking and binding antibody to IF, and antibody to gastric parietal cell. This observation suggests that LCT may reflect the degree of autoimmune derangement in pernicious anemia, particularly since LCT was not detected in any of the 12 sera that contained none of the three autoantibodies. The patients with LCT in the present study did not appear to differ clinically from those without LCT. The presence of cytotoxins in pernicious anemia could not be attributed to immunization with allogeneic lymphocytes for several reasons; none of the patients with LCT gave a history of transfusion, and all cytotoxins were detected at 15°C and not at 24°C. Earlier studies have suggested that lymphocytotoxins induced by allogeneic immunization, such as fetal-maternal transfer, react at 24°C, whereas those associated with viral disease or autoimmunity are detected primarily at 15°C.

The etiology and possible biologic significance of cold-reactive LCT remain to be determined. The presence of these cytotoxins in certain autoimmune diseases is particularly intriguing, since in these conditions they may be a gauge of immune derangement, could be related to a causal agent, or might represent a defense mechanism, i.e., a naturally occurring immunosuppressant.

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Lymphocytotoxins and Pernicious Anemia

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